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Blocking effects of polyunsaturated fatty acids on Na^+ channels of neonatal rat ventricular myocytes

(Na^+ current/cardiomyocyte/eicosapentaenoic acid/antiarrhythmia/excitability–automaticity)

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ABSTRACT **Recent evidence indicates that polyunsaturated long-chain fatty acids (PUFAs) prevent lethal ischemia-induced cardiac arrhythmias in animals and probably in humans. To increase understanding of the mechanism(s) of this phenomenon, the effects of PUFAs on Na^+ currents were assessed by the whole-cell patch-clamp technique in cultured neonatal rat ventricular myocytes. Extracellular application of the free 5,8,11,14,17-eicosapentaenoic acid (EPA) produced a concentration-dependent suppression of ventricular, voltage-activated Na^+ currents (I_{Na}). After cardiac myocytes were treated with 5 or 10 μM EPA, the peak I_{Na} (elicited by a single-step voltage change with pulses from −80 to −30 mV) was decreased by 51% ± 8% ($P < 0.01$; $n = 10$) and 64% ± 5% ($P < 0.001$; $n = 21$), respectively, within 2 min. Likewise, the same concentrations of 4,7,10,16,19-docosahexaenoic acid produced the same inhibition of I_{Na}. By contrast, 5 and 10 μM arachidonic acid (AA) caused less inhibition of I_{Na}, but both $n - 6$ and $n - 3$ PUFAs inhibited I_{Na} significantly. A monounsaturated fatty acid and a saturated fatty acid did not. After washing out EPA, I_{Na} returned to the control level. Raising the concentration of EPA to 40 μM completely blocked I_{Na}. The IC_{50} of EPA was 4.8 μM. The inhibition of this Na^+ channel was found to be dose and time, but not use dependent. Also, the EPA-induced inhibition of I_{Na} was voltage dependent, since 10 μM EPA produced 83% ± 7% and 29% ± 5% inhibition of I_{Na} elicited by pulses from −80 to −30 mV and from −150 to −30 mV, respectively, in single-step voltage changes. A concentration of 10 μM EPA shifted the steady-state inactivation curve of I_{Na} by −19 ± 3 mV ($n = 7$; $P < 0.01$). These effects of PUFAs on I_{Na} may be important for their antiarrhythmic effect *in vivo*.**

During the period from 1950 to 1975, there were many animal studies *in vivo* and *ex vivo* reporting the effects of fatty acids on cardiac arrhythmias in an effort to define a possible role of increased concentrations of fatty acids as a cause of malignant arrhythmias induced by myocardial infarctions and ischemia. At the time, no distinction was made regarding the state of saturation or of unsaturation of the fatty acids. In 1981, Murnaghan (1) reported polyunsaturated long-chain fatty acids (PUFAs) were antagonistic to the depressing effects of hypoxia and of long-chain saturated fatty acids (SFAs) on the arrhythmia threshold of isolated rabbit hearts and concluded, "when polyunsaturated fat is consumed in the diet, it may . . . have a protective action against the development of cardiac arrhythmias. . . ." This conclusion has been put on an experimental base by McLennan *et al.* (2, 3) who followed up on sporadic earlier reports of antiarrhythmic effects of PUFA (1, 4). They have shown in several feeding studies in rats that, when fish oils constitute a major source of dietary fat, ischemia-induced malignant ventricular arrhythmias are prevented (2), and in marmosets the arrhythmia threshold is increased (3). In conscious, exercising, prepared dogs Billman *et al.* (5) were able to prevent ischemia-induced, fatal ventricular arrhythmias by an emulsion of fatty acids, largely 5,8,11,14,17-eicosapentaenoic acid [EPA; C20:5 ($n - 3$)] and 4,7,10,16,19-docosahexaenoic acid [DHA; C22:6 ($n - 3$)] infused intravenously just prior to inducing the ischemic stress, showing that it is indeed these $n - 3$ fatty acids that are the antiarrhythmic component of fish oils. Further studies from our laboratory have demonstrated in isolated neonatal rat cardiac myocytes the following: (*i*) PUFAs with two or more unsaturated carbon-carbon bonds of both the $n - 6$ and $n - 3$ class have antiarrhythmic properties, but certain cyclooxygenase and lipoxygenase eicosanoids of arachidonic acid (AA) are arrhythmogenic (6); (*ii*) these fatty acids are promptly antiarrhythmic only as the free fatty acid, and their effect does not require incorporation into membrane phospholipids or covalent bonding to other membrane constituents (6); (*iii*) these fatty acids reduce the electrical excitability of cardiac myocytes by hyperpolarizing the resting or diastolic membrane potential and increasing the threshold for the gating of the fast Na^+ channel. They also markedly prolong the relative refractory period of the myocytes (7). As an initial effort to understand the ionic bases of these electrophysiologic effects of the PUFAs, we report their inhibitory effect on Na^+ currents, I_{Na}, of cultured neonatal rat cardiac myocytes.

MATERIALS AND METHODS

Materials. All fatty acids were obtained from Sigma. Fatty acids were dissolved weekly in ethanol at a concentration of 10 mM and stored under a nitrogen atmosphere at −20°C before use. The experimental concentration of fatty acids was obtained by dilution of the stocks and contained negligible concentrations of ethanol, which alone has no effect on ventricular Na^+ currents. The pipette solution for recording the inward Na^+ current contained 100 mM CsCl, 40 mM CsOH, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, 10 mM Hepes, and 5 mM MgATP (pH adjusted to 7.3 with CsOH). In all experiments (except as indicated in the text) the bath solution contained 40 mM NaCl, 100 mM *N*-methyl-D-glucamine, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, and 10 mM glucose (pH adjusted to 7.4 with HCl).

Cell Culture. Primary cultures of ventricular myocyte were prepared from 1-day-old neonatal rats with a commercial isolation kit (Worthington). This kit utilizes purified enzyme preparations to produce healthy beating cells, which were seeded at a density of 3×10^5 cells per ml. Cells were incubated at 37°C in air/5% CO_2 and 98% relative humidity (model 3123,

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Abbreviations: EPA, 5,8,11,14,17-eicosapentaenoic acid; DHA, 4,7,10,16,19-docosahexaenoic acid; AA, arachidonic acid (5,8,11,14-eicosatetraenoic acid); PUFA, polyunsaturated long-chain fatty acid; SFA, saturated fatty acid; ETYA, 5,8,11,14-eicosatetraynoic acid.

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Forma Scientific, Marietta, OH). The culture medium was changed every other day. Ventricular myocytes used for patch-clamp experiments were cultured for 3–6 days.

Recording of Cardiac Na^+ Current. A glass coverslip seeded with ventricular myocytes was carefully transferred to a chamber containing 0.5 ml of bath solution mounted on an inverted microscope (Nikon). Cells were continuously superfused (1–2 ml/min) with a Tyrode solution containing 137 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, and 10 mM glucose (pH 7.4). Recording pipettes had a resistance of 2–5 MΩ and were connected via a Ag–AgCl wire to an Axopatch 1D amplifier (Axon Instruments, Burlingame, CA). After forming a conventional gigaseal, the capacitance of an electrode was compensated. Additional suction was used to form whole-cell configuration (8). In some ventricular myocytes whole-cell membrane capacitance was measured as described (9). The average membrane capacitance of 36 single cells was 58 ± 4 pF. Correction of cell capacitance and series resistance was then performed before application of an experimental voltage-clamp protocol. Currents were stored in a personal computer running PCLAMP 5.5.1 data acquisition. Recordings of I_{Na} were made in the same myocyte before, during, and after exposure to PUFA in the presence of 1.0 μM nifedipine in the bath solution to block the L-type Ca^{2+} channel. External solutions were exchanged by fast application by using a modified puffer-pipette system (10). Experiments were performed at room temperatures (22–23°C).

Statistics. Data from two groups were analyzed by analysis of variance (ANOVA) or by paired or unpaired Student's *t* test. $P < 0.05$ was taken to indicate a significant difference. All data are presented as mean ± SEM.

RESULTS

Voltage-Dependent Na^+ Currents in Cultured Neonatal Rat Ventricular Myocytes. Most cultured single ventricular myocytes were round with a diameter of 10–25 μm. The average capacitance of the ventricular myocytes was 58 ± 4 pF (n = 36). Generally, voltage-dependent Na^+ currents could be recorded in spontaneously beating ventricular myocytes (2- to 6-day cultures). In more than 50% of nonspontaneously beating cells, this voltage-activated Na^+ current was not observed. The absence of I_{Na} in nonspontaneously beating cells may be due to damage to the cell membrane during isolation or the presence of other nonexcitable cells. The current densities elicited from −80- to −30-mV pulses were −39 ± 4 and −80 ± 21 pA/pF for extracellular concentrations of Na^+ of 40 (n = 27) and 137 (n = 4) mM, respectively.

EPA-Induced Suppression of I_{Na}. Fig. 1 *Upper* shows 5 μM EPA-induced suppression of superimposed Na^+ currents recorded from a cultured ventricular myocyte of a neonatal rat. The EPA-induced suppression of I_{Na} did not alter the current–voltage relations (Fig. 1 *Lower*). The cardiac Na^+ current was activated at −60 to −50 mV and achieved a peak amplitude at −30 mV in the control and also in the presence of EPA (n = 6).

In most cells, EPA-induced inhibition of I_{Na} returned to the control level after EPA was washed out with bath solution containing 1–2 mg of bovine serum albumin per ml. If the washing procedure was started promptly before the maximal inhibition was reached, bath solution alone was used to wash out the suppressant effect of EPA on I_{Na}. Fig. 2 is an example of an I_{Na} which recovered from EPA-induced suppression after washing with the bath solution alone. The inhibitory effect of EPA on cardiac I_{Na} began within 10 s and reached a new steady-state level within 2 min after puffing 5–10 μM EPA. EPA did not significantly alter the activation and inactivation time constants of I_{Na} calculated by least-squares fitting of the single exponential. The mean values (n = 10) of activation and inactivation time constants of I_{Na} elicited from −80 to −30 mV were 0.57 ± 0.09 and 2.8 ± 1.0 ms for the control and 0.54 ± 0.12 and 3.6 ± 1.1 ms for the EPA treatment, respectively (values are not significantly different).

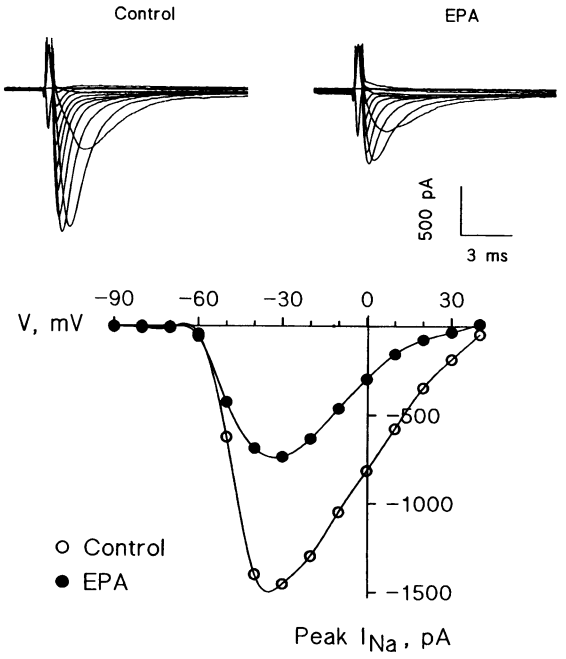

FIG. 1. EPA suppresses the voltage-activated Na^+ current (I_{Na}) in a representative cultured neonatal rat ventricular myocyte. (*Upper*) Superimposed traces in the absence (Control) and presence (EPA) of 5 μM EPA. The currents were elicited by 10-mV increment voltage steps (20 ms; 0.2 Hz) from a holding potential of −80 mV down to −90 mV and up to 40 mV. (*Lower*) Current–voltage relations of peak I_{Na} in the absence (○) and presence (●) of 5 μM EPA.

A similar series of experiments was done at a physiological concentration of 137 mM Na^+. The average peak I_{Na} elicited from −80 to −30 mV was −4542 ± 1241 pA with an extracellular Na^+ concentration of 137 mM (data not shown). Superfusion with 10 μM EPA suppressed the I_{Na} to −1443 ± 438 pA (n = 4; $P < 0.05$), a 68% inhibition. The current–voltage relations, the onset of the inhibitory effect of EPA on the peak I_{Na}, reversal of EPA-induced supression by washing with bath solution containing 1–2 mg of bovine serum albumin per ml, and the time course of EPA washout were the same with extracellular Na^+ concentrations of 137 and 40 mM.

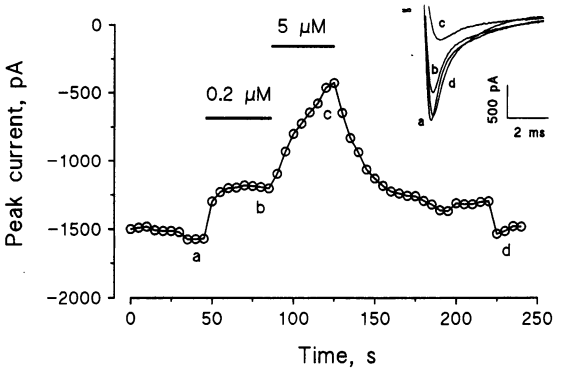

FIG. 2. Time course of the inhibitory effect of EPA on the peak I_{Na} recorded from a cultured neonatal rat ventricular myocyte bathed with 40 mM Na^+. The short lines marked with 0.2 μM and 5 μM represent the duration of extracellular application of 0.2 μm and 5 μM EPA. (*Inset*) Original current traces recorded by 20-ms pulses from a holding potential of −80 mV to −30 mV in a single step every 5 s. a, b, c, and d are the currents picked from the corresponding points of the time course marked with the same characters.

Fig. 3 summarizes the concentration dependence of the EPA-induced suppression of I_{Na} in cultured neonatal rat ventricular myocytes. The apparent IC_{50} of EPA was 4.8 μM. I_{Na} was almost completely inhibited by 40 μM EPA.

Voltage- and Time-Dependent Blocking Effect of EPA on I_{Na}. Fig. 4 shows that EPA modified the voltage dependence of the steady-state inactivation of I_{Na} in these myocytes. The membrane potential of this cell was held at −80 mV. Currents were elicited with a double-pulse protocol which consisted of a 30-ms testing pulse to −30 mV following a 500-ms conditioning prepulse varying from −140 mV to −30 mV in 10-mV increments every 10 s (Fig. 4*A*). Fig. 4*B* indicates that extracellular application of 10 μM EPA virtually suppressed I_{Na} at prepulses positive to −80 mV, but suppression was <50% with prepulse voltages of −120 to −140 mV. $V_{1/2}$ for the normalized steady-state inactivation curve of peak Na^+ currents shifted by −28 mV after exposing the myocyte to the solution containing 10 μM EPA in this experiment. A similar effect of EPA on the steady-state inactivation was also observed in another six cells; the shift averaged −19 ± 3 mV for seven myocytes ($P < 0.01$). Fig. 4*C* summarizes the voltage-dependent inhibition of I_{Na} with various prepulses in seven ventricular myocytes treated with 10 μM EPA. To further test the voltage-dependent suppression of EPA on I_{Na}, the holding potential was set at either −150 mV or −80 mV (Fig. 5) in four ventricular myocytes. The peak Na^+ currents were induced by 20-ms depolarizing pulses from either −80 or −150 mV to −30 mV in single-step voltage changes. Extracellular application of 10 μM EPA produced 83% ± 7% and 29% ± 5% inhibition of I_{Na} compared with its own control for the holding potential of −80 and −150 mV, respectively. These results demonstrate that EPA affects the voltage-gated parameters of cardiac I_{Na}.

To understand whether the EPA-induced blocking effect of I_{Na} was use dependent or time dependent, we set different rates of depolarizing pulses to observe the effects of time after exposure to EPA versus frequency of stimulation toward attaining the maximal suppression of I_{Na} for a given concentration of EPA. Na^+ currents were elicited by a train of depolarizing pulses from a holding potential of −80 mV to −30 mV following a 1- to 2-min rest. Application of a train of stimulating pulses at frequencies of 1.0, 0.2, 0.1, or 0.03 Hz had no effect on the time required to attain the same level of inhibition of I_{Na} at the concentration of EPA used, whether the concentration was 5 μM ($n = 5$) or 10 μM ($n = 5$). Thus, block of I_{Na} by EPA is time dependent not use dependent.

Effects of Other PUFAs and SFAs on I_{Na}. Table 1 summarizes the effects of DHA, α-linolenic acid [C18:3 ($n - 3$)], AA [C20:4 ($n - 6$)], linoleic acid [C18:2 ($n - 6$)], the oleic acid [C18:1 ($n - 9$)], and stearic acid (C18:0) on I_{Na} of cultured neonatal rat ventricular myocyte. The pattern of percent inhibition of I_{Na} shown by these fatty acids was the same pattern as that shown by their antiarrhythmic actions (6) and their effects on the electrophysiology of the cardiac myocytes (7). Both the $n - 6$ and $n - 3$ classes of essential PUFAs caused major inhibition of I_{Na}, with AA seemingly less potent than the others. ETYA, the AA analog with four triple C≡C bonds again acted like the other PUFAs in inhibiting I_{Na}. By contrast, the monounsaturated fatty acid oleic acid and the SFA stearic acid had no significant inhibitory effects on I_{Na}, again consistent with their lack of antiarrhythmic activity (6).

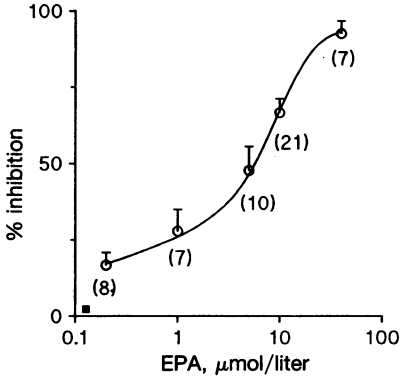

FIG. 3. The dose–response curve of EPA suppression of I_{Na}. Peak Na^+ currents were obtained by the same single-step protocol described in the legend to Fig. 2 (−80 to −30 mV) for both the control and inhibited I_{Na} at each concentration of EPA. The number of ventricular myocytes treated at each concentration of EPA is indicated in parentheses. The IC_{50} of EPA is 4.8 μM.

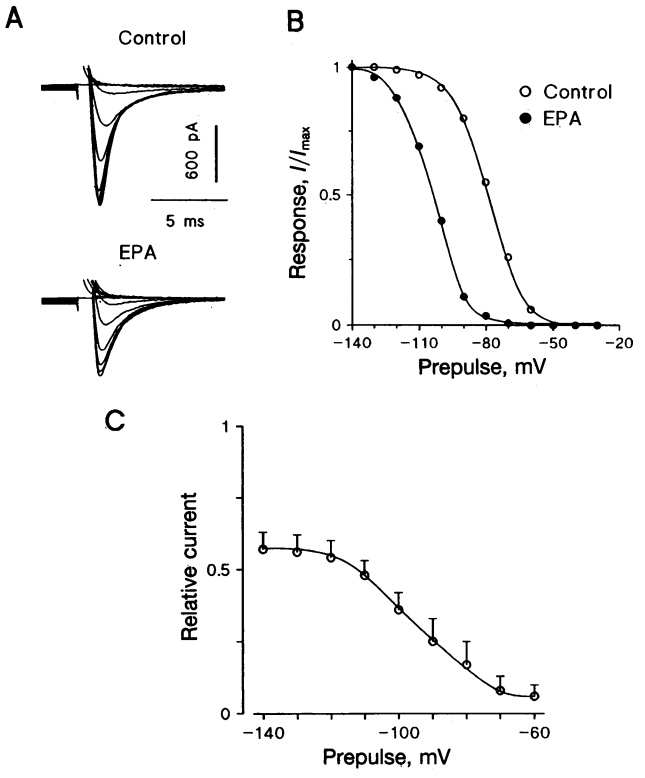

FIG. 4. EPA modifies the voltage dependence of the steady-state inactivation of Na^+ channels of neonatal rat cardiac myocytes. (*A*) Superimposed original currents recorded in the absence (control) and presence of 10 μM EPA. Currents were elicited with a double-pulse protocol consisting of a 30-ms testing pulse to −30 mV following a 500-ms conditioning prepulse varying from −140 to −30 mV in increments of 10 mV every 10 s from a holding potential of −80 mV. (*B*) Normalized steady-state inactivation of peak Na^+ currents as a function of the prepulse voltages with the protocol of *A*. Extracellular application of 10 μM EPA suppressed I_{Na} at prepulses positive to −80 mV. $V_{1/2}$ for the normalized steady-state inactivation curve of the peak Na^+ currents was shifted to the left by EPA. (*C*) Prepulse voltage-dependent suppression of 10 μM EPA on cardiac Na^+ currents ($n = 7$). Values along the x axis represent different voltages of the conditional prepulses. The values along the y axis represent the relative peak Na^+ currents produced by depolarizing the membrane to −30 mV from various voltages of the conditional prepulses. The relative currents were calculated as $I_{Na(EPA)}/I_{Na(control)}$ from the same corresponding prepulse voltage.

DISCUSSION

Consistent with our previous studies, the isolated neonatal rat cardiac myocyte preparation was used for these studies. This preparation is convenient because, unlike the adult rat heart cell, it beats spontaneously and rhythmically after 3 to 5 days in culture medium. However, because of well-documented changes in ion channels with maturation of the heart and because of species differences as to which ion channels are

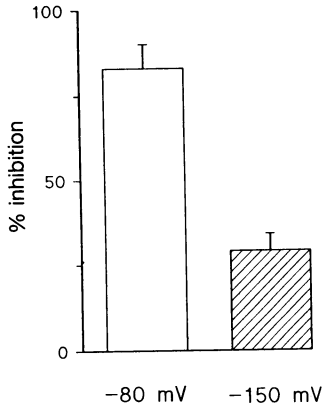

FIG. 5. Comparison of the inhibitory effects of 10 μM EPA on I_{Na} elicited by pulses from holding potentials of either −80 or −150 mV to −30 mV, each as single-step voltage changes. The relative currents were calculated as described in the legend to Fig. 4.

dominant, we are aware that the response of these cells to PUFAs may differ from the responses of hearts of other species and of animals of different ages. Our aim in this study was to explain the dramatic effects of these PUFAs on the electrophysiology of this cell preparation.

This study demonstrates a prompt inhibitory action of PUFA on the Na^+ currents through fast sodium channels responsible for the phase 0 of the action potential in isolated neonatal rat cardiac myocytes. The inhibition of this ion channel was dose, time, and voltage dependent, but not use dependent. An inhibitory effect on this sodium channel was expected from our earlier observations on the electrophysiologic effects of PUFAs, which showed a more positive electrical threshold for the gating of the Na^+ channel in the presence of these fatty acids (7). Similarities between the effects of PUFAs, lidocaine, and tetrodotoxin on the spontaneous beating rate of the isolated myocyte and on the levels of cytosolic free Ca^{2+} in the presence of tetrodotoxin also suggested that Na^+ channels would be modulated by PUFAs (1, 11). Tetrodotoxin has been reported also to produce a voltage- and time-dependent block of cardiac sodium channels in guinea pig ventricular muscles (12).

Table 1. Comparison of the blocking effects of fatty acids on Na^+ channels in cultured neonatal rat ventricular myocytes

Fatty acid	Structure	% inhibition of I_{Na} 5 μM	% inhibition of I_{Na} 10 μM
n − 3 PUFA			
EPA	20:5 (*n* − 3)	51 ± 8* (10)	64 ± 5† (21)
DHA	22:6 (*n* − 3)	52 ± 8* (7)	66 ± 11‡ (5)
LNA	18:3 (*n* − 3)	—	71 ± 5‡ (5)
n − 6 PUFA			
AA	20:4 (*n* − 6)	13 ± 5 (6)	44 ± 14‡ (5)
LA	18:2 (*n* − 6)	—	62 ± 11‡ (5)
ETYA	20:4	—	52 ± 9‡ (6)
Monounsaturated			
OA	18:1 (*n* − 9)	—	9 ± 8 (7)
SFA			
SA	18:0	4 ± 5 (6)	8 ± 6 (4)

Values are expressed as the mean percent inhibition of I_{Na} ± SEM. Numbers in parentheses are the numbers of individual cells treated with the fatty acids. Statistical significance was tested between the peak amplitudes of I_{Na} suppressed by fatty acids and the corresponding controls. ETYA, 5,8,11,14-eicosatetraynoic acid, an AA analog but with four carbon-carbon triple bonds rather than double bonds; LNA, α-linolenic acid; LA, linoleic acid; OA, oleic acid; SA, stearic acid.
*$P < 0.01$.
†$P < 0.001$.
‡$P < 0.05$.

The rate of washout of the inhibition of I_{Na} seemed quite rapid even without the presence of albumin, as shown in Fig. 2. As noted, this rapid reversibility was observed only if the contact of the myocyte with the free PUFA is brief. Had the inhibition of I_{Na} been sustained to attain its full steady-state level, the washout and reversal of the extent of inhibition would have been much slower and required superfusion with delipidated bovine serum albumin. The explanation for the early rapid-washout effect is not clear, but the fatty acids partitioning into the phospholipids of the sarcolemma might be moving with time into an intracellular compartment from which their removal by washout is slower.

The voltage-dependent inhibition of I_{Na} could prove helpful in preventing arrhythmias during ischemia. Myocytes that are partially depolarized are the cells least stable electrically and, thus, most likely to respond to random, small, depolarizing stimuli. In the presence of these antiarrhythmic PUFAs and the resultant inhibition of I_{Na}, the partially depolarized myocytes may have their electrical stability increased. Fully polarized myocytes will have the smallest inhibition of I_{Na}, and their electrical stability should be least affected.

Although we cannot make definite statements without single-channel measurements, our results seem consistent with an effect of PUFAs to prolong the inactive state of the Na^+ channels. Several compounds have been reported to modify Na^+ channels by prolonging the inactivated states of Na^+ channels and, thus, to reduce excitability. Williams (13) indicated that class I antiarrhythmic drugs interfered with the process by which Na^+ channels were reactivated in response to repolarization and extended the effective refractory period to a point long after the time at which repolarization was already complete. Prolonging the inactivated state of the myocytes could account for the long relative refractory period induced by PUFAs despite a reduced action-potential duration.

Phenytoin, an anticonvulsant, has been reported to interact with inactive states of sodium channels of neuroblastoma cells to reduce the average open time of Na^+ channels (14). This suggested that the compound can bind to Na^+ channels and produce a long-duration, nonconducting state for which the probability of a channel opening is small. These modifications could underlie the selective block of action potentials during chronic depolarization of the membrane or during high-frequency discharges, as we have observed in the antiarrhythmic actions of PUFAs. Also, dihydropyrazole insecticides produce a voltage-dependent block in axons described as a uniform shift of the steady-state (slow) sodium inactivation curve in the direction of hyperpolarization indicative of selective binding to inactivated states of the channel (15).

Further, the shift by −19 mV of the steady-state inactivation curve of I_{Na} by EPA could be consistent with an effect of the negative charge contributed from the free carboxyl head group of the PUFA to increase the density of negative charges near the voltage sensor of Na^+ channels. This effect might be analogous to that reported by Perzo and Bezanilla (16) for the effect of phosphorylation, which increases the density of negative surface charges in the vicinity of the voltage sensor of the delayed rectifier K^+ channel and shifts its voltage dependence toward more depolarized potentials. We have shown that esterified EPA or DHA does not have prompt antiarrhythmic effects on isolated neonatal rat heart cells or canine hearts *in vivo*; only the free fatty acids are effective (5, 6). Perhaps the charge of the free carboxylic fatty acid head group could account for the more positive threshold potential for the gating of the fast Na^+ channel in the presence of the free PUFAs, as we have found (7).

Inhibitory effects on myocardial Na^+ channels are features of other clinically used antiarrhythmic drugs of the class I type. Two drugs with class IC antiarrhythmic action, encainide and flecainide, were tested in the cardiac arrhythmia suppression trial (CAST) and were found to increase cardiac mortality (17).

A possible explanation for the deleterious effects during chronic administration of these class IC antiarrhythmic drugs, despite their suppression of ventricular ectopy with short-term use, comes from the finding that inhibition of the fast Na^+ channels by class I antiarrhythmic agents leads to upregulation of sarcolemma Na^+ channels (18, 19). Whether the inhibition of the fast Na^+ channel by PUFAs also will result in upregulation of the Na^+ channel α subunits is not known, but present evidence in rats (2), marmosets (3), and humans (20, 21) receiving prolonged treatment with $n - 3$ PUFA is that PUFAs are antiarrhythmic not arrhythmogenic. Furthermore, other features of the electrophysiologic effects of PUFAs (7) also suggest that ion channels other than the fast Na^+ channels are modulated by PUFAs. An increased activity of some outward K^+ channel might explain the observed hyperpolarization of the resting or diastolic membrane potential, as well as the reduced duration of the action potential. The latter, however, might also result from some inhibition of the L-type Ca^{2+} channels.

There are published studies examining the effects of certain PUFAs on these channels, but the reports seem contradictory at this time. Outward-rectifying potassium channels have been reported to be activated by PUFA (22), but inhibition of outward channels has also been reported (23, 24). The situation regarding the effects of these PUFAs on calcium channels is also conflicting at this time (25, 26). The role of PUFAs in modulating Ca^{2+} channels also needs further study.

As an initial effort to define the ionic basis of the marked electrophysiologic effects of PUFA on cultured neonatal rat cardiac myocytes, we have demonstrated a definite block of the fast Na^+ channels by low concentrations of PUFA. Other ion channels need to be carefully examined before the antiarrhythmic effects of the PUFA are explained. Then the means by which the PUFA affect the ion channels will still need to be determined.

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